CHALLENGES ASSOCIATED WITH THE ANALYSIS OF MONO-SUBSTITUTED POLYFLUORINATED PHOSPHATE ESTERS BY LCMS

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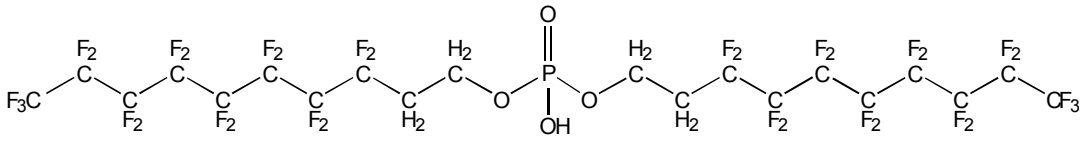
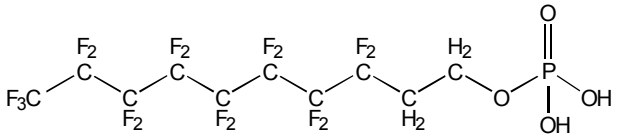
Introduction

Polyfluorinated phosphate esters can be mono-, di-, or tri-substituted (monoPAPs, diPAPs or triPAPs respectively) and the polyfluorinated alkyl chain can vary in length with the most commonly observed homologues being the 6:2 [$CF_3(CF_2)_5(CH_2)_2$] and 8:2 [$CF_3(CF_2)_7(CH_2)_2$] chains. It has been shown that compounds such as PAPs can leach out of food packaging and migrate into the food that we ingest.[1,2,3] Although the phosphate ester linkages for both mono- and di-PAPs have been shown to be stable to abiotic hydrolysis[4], cleavage can still occur within biological systems producing fluorinated alcohols which would likely be metabolized to perfluoroalkylcarboxylic acids[5]. Various methods have been developed and validated for the analysis of poly- and per-fluorinated compounds, however the applicability of these methods to mono- and di-substituted phosphate esters appears to be limited. The work presented here outlines some challenges that may be encountered during the analysis of the monoPAPs as well as possible solutions.



8:2 diPAP



8:2 monoPAP

Materials and Methods

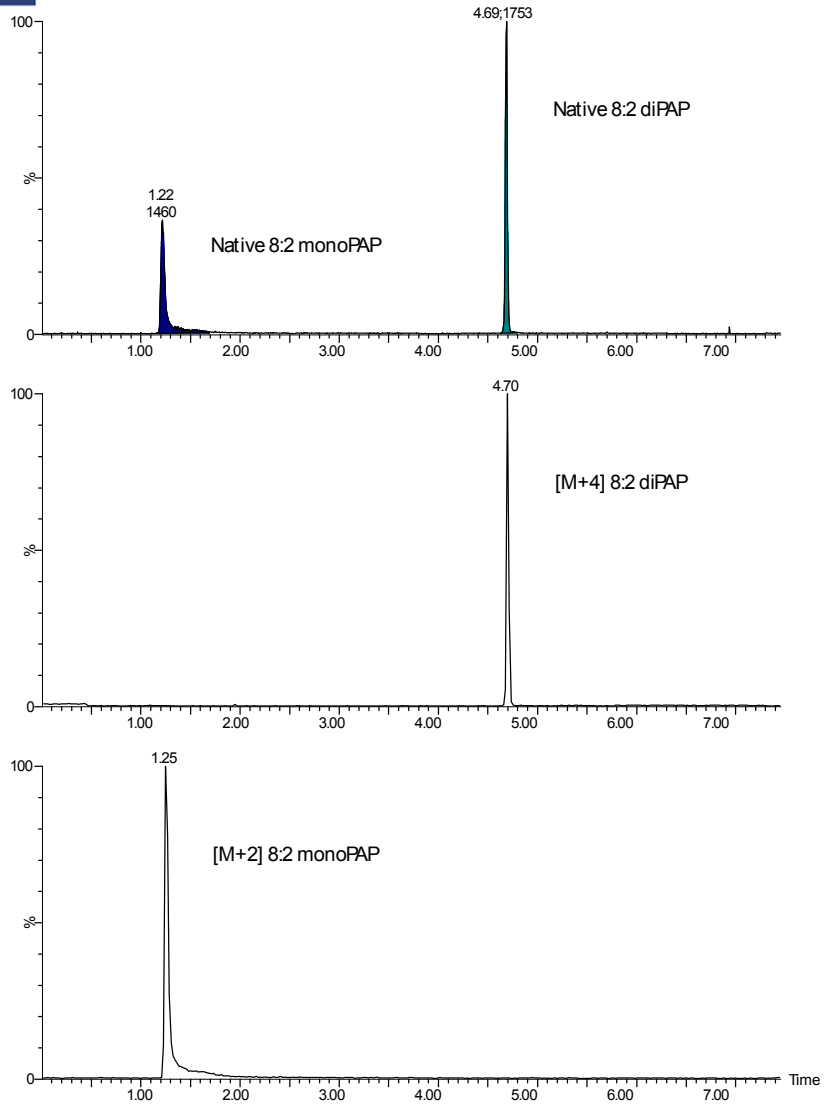
The native 8:2monoPAP, native 8:2diPAP, mass-labelled [M+2]8:2monoPAP ($^{13}C_2$), and mass-labelled [M+4]8:2diPAP ($^{13}C_4$) were synthesized at Wellington Laboratories Inc. (Guelph, ON) using proprietary methods. The native 6:2monoPAP and native 6:2diPAP were received from Scott Mabury's research group (University of Toronto, Toronto, ON).

Characterization of all of the polyfluorinated phosphate esters was accomplished using a combination of ^{1}H NMR, ^{19}F NMR, ^{31}P NMR and LC/MS/MS analysis. LC/MS experiments were conducted on a Waters Acquity Ultra Performance LC interfaced to a Micromass Quattro micro API (triple quad mass spectrometer).

Separations were performed on a Waters Acquity BEH C_8 column (1.7 um, 2.1 x 100 mm). MS data were collected in MRM or full scan mode (capillary voltage = 3.00 kV; cone voltage = 35.00 V). All LCMS samples were prepared using 75:25 methanol:water (pH 11) as the reconstitution solvent. NMR experiments were performed on an Avance-400 MHz Bruker instrument.

Mobile Phase A	Water (pH 11 adjusted with ammonium hydroxide)
Mobile Phase B	Methanol
Flow	0.300 ml/min
Initial (%B)	0 min (60% B)
Time 1 (%B)	5 min (90% B)
Time 2 (%B)	8.5 min (90% B)
Time 3 (%B)	9 min (60%B)

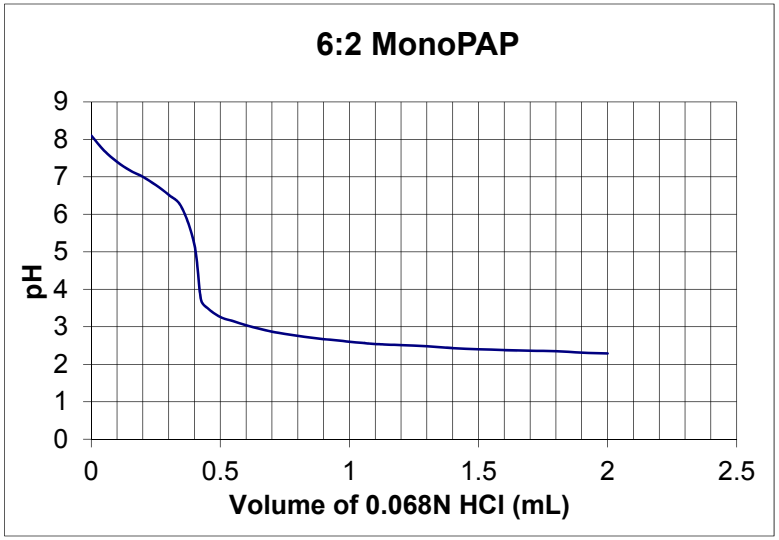
Results and Discussions

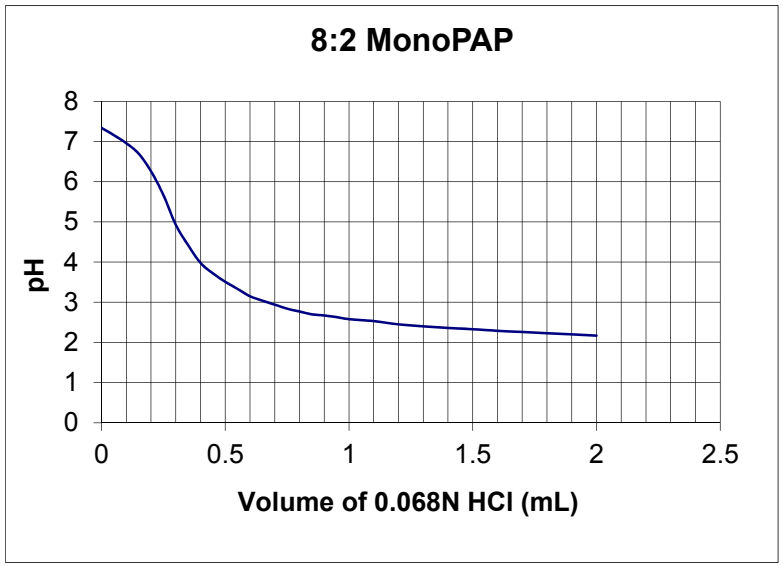


Perfluoroalkylphosphonates are known to form mono-layers on the surfaces of solid substrates such as metals and glass[6]. The affinity of these compounds to metal surfaces has also resulted in peak tailing during liquid chromatography. It is possible to improve peak shape and form the mono-anion by utilizing a relatively high pH in the reconstitution solvent and at the beginning of the gradient and then slowly buffering the pH over the course of the gradient.

Initially, LCMS analysis of the polyfluorinated phosphate esters was attempted using conditions similar to those previously developed for perfluoroalkylphosphonic acids (PFAPAs). Peaks were observed for the 6:2 mono- and di-PAPs as well as the 8:2diPAP, but the 8:2monoPAP eluted from the column as an extremely broad “lump” instead of a sharp peak. A number of mobile phase combinations were evaluated and it was determined that the response of the monoPAPs could be optimized and peak tailing minimized by utilizing a methanol:water gradient when the pH of the water was adjusted to 11 with ammonium hydroxide (see Table on slide 3).

Results and Discussions



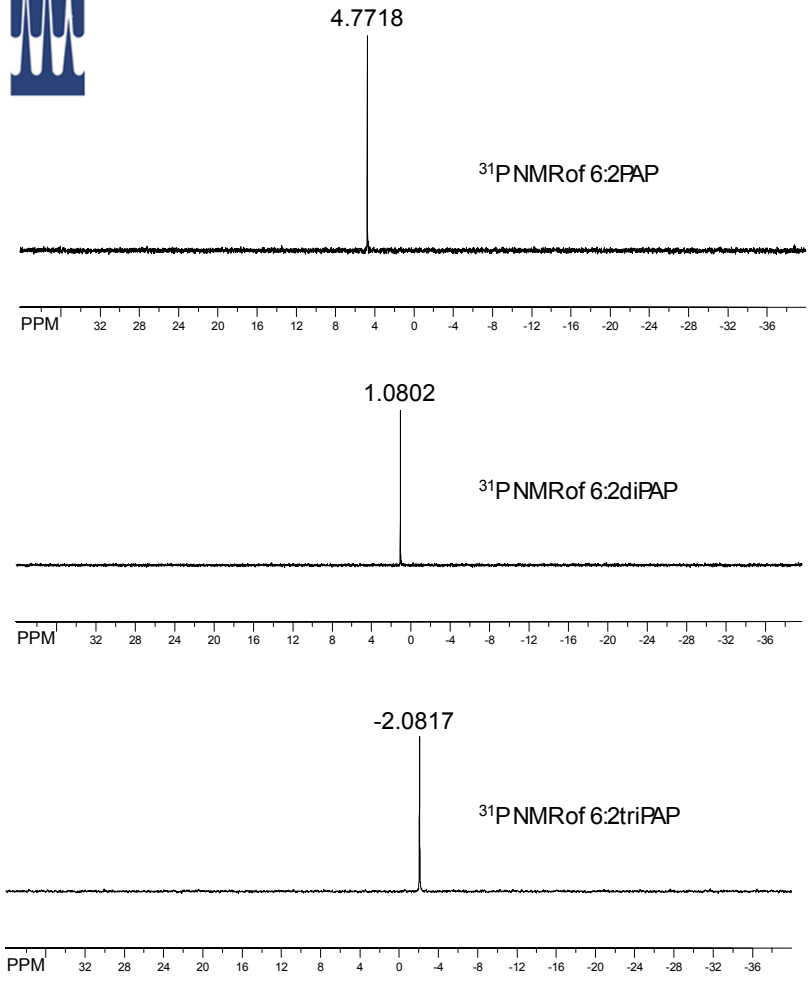


We initially believed that because a higher pH was required to minimize peak tailing and observe the mono-anion of these compounds, the pKa's of the PAPs were substantially higher than those previously determined for the PFAPAs. Determination of the pKa's for the 6:2monoPAP and 8:2monoPAP resulted in values that were comparable to those of mono-n-alkyl phosphates.

6:2monoPAP: pK_{a1} = 3.00, pK_{a2} = 6.95
8:2monoPAP: pK_{a1} = 3.20, pK_{a2} = 6.30

Interesting aggregation behaviour has been documented for mono-n-alkyl phosphates with branched isomers forming vesicles even at high pH[7]. The incorporation of fluorine into the molecule should amplify this behaviour by increasing the hydrophobicity of the phosphate tail. It is possible that a pH of 11 was required in the mobile phase in order to suppress the formation of aggregates. The addition of a buffer could also cause problems by creating a "salting out" effect that would promote the formation of vesicles and limit ionization.

Results and Discussions



REFERENCES

1. Begley TH et al. (2005); *Food Addit. Contam.* 22(10): 1023-1031
2. Begley TH et al. (2007); *Food Addit. Contam.* 25(3): 1-7
3. Lee H et al. (2010); *Environ Sci. Technol*. 44(9): 3305-3310
4. D'eon JC, Mabury SA. (2007); *Environ Sci. Technol*. 41(13): 4799-4805
5. D'eon JC, Mabury SA. (2011); *Environ Health Perspect.* 119(3): 334-350
6. Boardman LC, Pellerite MJ. 3M Innovative Properties Company (2004) *Fluorinated Phosphonic Acids*, U.S. Pat. 6,824,822 B2
7. Walde P et al. (1997); *J. Phys. Chem. B.* 101: 7390-7397

In fact, the addition of sodium hydroxide to the 6:2monoPAP completely suppressed the signal when the solution was infused directly into the mass spectrometer. Interestingly, the addition of ammonium hydroxide resulted in an increase in signal strength. This could be the result of the ion-pair surviving the electrospray ionization process or micelle formation. Either way, this property could also have an effect on the extraction efficiency of these compounds from certain matrices.

The utilization of clean mono- and di-substituted polyfluorinated phosphate esters, as well mass-labelled internal standards, will aid in the development of quantitative analytical methods. The ^{31}P NMR spectra showing the characteristic shifts of the 6:2mono-, di- and tri-PAPs are shown on the left. Although much of the unique behaviour associated with perfluorinated compounds can be attributed to their poly- or per-fluorinated alkyl chains, it is evident that the functional groups associated with each sub-class can have a large impact on the ease of their analysis.